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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/520,693	01/20/2006	Alastair Dixon	GJE-7135	9643
23557 7590 09/18/2008 SALIWANCHIK LLOYD & SALIWANCHIK A PROFESSIONAL ASSOCIATION PO BOX 142950 GAINESVILLE, FL 32614-2950				
EXAMINER WOOLWINE, SAMUEL C				
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary

Application No.

10/520,693

Applicant(s)

DIXON ET AL.

Examiner

SAMUEL WOOLWINE

Art Unit

1637

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 07 July 2008.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-20 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-20 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- 1) ☐ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO/SF/ICE)
Paper No(s)/Mail Date _____
- 4) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date _____
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: _____

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 07/07/2008 has been entered.

Status

Claims 1-20 are pending in the application. Other than newly added claim 20, the claims have not been amended since being finally rejected in the Office action mailed 03/05/2008.

The rejection of claims 1-6 and 8-19 under 35 U.S.C. 103(a) over Richardson et al (WO 01/06004 A2) in view of Gu et al (US 2003/0180737) made in the Office action mailed 03/05/2008 is maintained for the reasons of record and reiterated below. The rejection is also extended to new claim 20, which is drawn to the same invention.

The rejection of claim 7 over these references and further in view of Fend et al (American Journal of Pathology 154(1):61-99; 1999) is also maintained for the reasons of record and reiterated below.

Applicant's remarks will be addressed following the rejections.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-6 and 8-20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Richardson et al (WO 01/06004 A2, cited on the IDS of 8/28/2006) in view of Gu et al (US 2003/0180737).

With regard to claim 1, Richardson teaches a *method for increasing the number of polynucleotides containing sequences corresponding to a mRNA species present in a sample* (e.g. page 25, lines 27-29), *the method comprising the steps of:*

(i) *reverse transcribing the mRNA species using a heeled 5'-amplification primer (FAP-RAND)* (e.g. "first heeled primer population"; page 25, lines 30-31)

and a heeled 3'-amplification primer (TAP-RT) (e.g. "second heeled primer population"; page 25, lines 32-33),

wherein each primer sequence is unique (it is noted in paragraph [0007] of Applicant's published application: "[a]nother advantage is that the production of complex products is minimised, due in part to the use of unique sequences in FAP and TAP which are absent from the genome being investigated"; however, Richardson clearly teaches this concept in, for instance, Example V, pages 55-58, wherein the primer sequences were chosen based on their absence from the genome being investigated (see page 55, lines 29-30 and page 56, lines 25-26; also note that Richardson teaches that the heel sequence of the first heeled primer "is not complementary to the first strand cDNA nor the mRNA molecules initially present in the sample" (page 39, lines 13-15) and that the heel sequence of the second heeled primer "is not complementary to the mRNA molecules present in the sample or with the first strand cDNA molecules synthesized at step a)", thus the heel sequences are "unique"),

and either or each heel sequence includes a RNA polymerase promoter site (see for example page 39, lines 16-17 (for the "first heeled primer") and page 40, lines 29-31 (for the "second heeled primer"); note also Richardson's Example V uses a T7 RNA polymerase promoter sequence for the first primer (page 55, lines 24-33) and a T3 RNA polymerase promoter sequence for the second primer (page 56, lines 24-31)),

and the FAP includes a variable sequence (Richardson teaches each primer as having a variable sequence; see page 39, lines 19-22 (for the first heeled primer) and page 40, lines 20-22 (for the second heeled primer)),

whereby the RNA is reverse-transcribed to produce double-stranded cDNA and then multiple cDNAs according to the variable sequence (page 25, lines 32-33 and page 26, line 21 through page 28, line 2; page 39, lines 19-22; page 40, lines 20-22; it is clear that the products synthesized are determined in part by the variable sequences present in the heeled primers);

and (ii) of amplifying the cDNA using primers sufficiently complementary to the primer sequences FAP and TAP, within FAP-RAND and TAP-RT (26, lines 4-7; page 31, line 26 through page 32, line 2).

With regard to claim 2, Richardson teaches *in vitro* transcription (see page 37, lines 12-25 and page 38, lines 5-24, for example).

With regard to claim 3, Richardson's Example V uses a T7 RNA polymerase promoter sequence for the first primer (page 55, lines 24-33) and a T3 RNA polymerase promoter sequence for the second primer (page 56, lines 24-31).

With regard to claim 4, see page 38, lines 5-24. Richardson teaches incorporation of an RNA polymerase promoter in the [first heeled] primer allows synthesis of complementary RNA, whereas incorporation of an RNA polymerase promoter in the second heeled primer allows synthesis of sense RNA. Either of these embodiments generates a strand-specific library.

With regard to claim 5, Richardson teaches using the method for the production of a subtracted library (page 44, lines 6-17; the "two cell populations" is implied by the "two different samples" at line 13).

With regard to claim 6, Richardson teaches immobilizing the polynucleotide products of his method to an array (page 37, lines 23-25). Note that there is no explicit definition of "cloning" in Applicant's disclosure. Therefore, the generation of multiple copies of a particular polynucleotide, as in the method of Richardson, can be considered "cloning".

With regard to claim 8, Richardson teaches using a sample from patch clamp harvesting (page 59, lines 14-28).

With regard to claims 9 and 10, Richardson teaches the first and second heeled primers having cleavage sites at the 3' ends of the heel sequences (page 39, lines 28-31 and page 40, lines 23-26).

With regard to claim 11, Richardson teaches the cleavage sites in the first and second heeled primers are identical (page 42, lines 25-27).

With regard to claim 12, Richardson teaches the cleavage sites in the first and second heeled primers are identical (page 42, lines 28-30).

With regard to claim 13, Richardson teaches an embodiment which includes an additional step of treating the polynucleotides with an agent (restriction enzyme) that cleaves at the cleavage site (page 35, lines 29-34).

With regard to claim 14, Richardson teaches up to 50 amplification cycles (page 31, lines 13-15).

With regard to claim 15, Richardson teaches each amplification cycle comprises the steps of obtaining single-stranded DNA molecules at a temperature between 80°C and 95°C (which overlaps the claimed range; page 32, lines 5-6), annealing the single-

stranded DNA molecules at a temperature between 65°C and 75°C (which overlaps the claimed range; page 32, lines 10-12), and elongating the annealed DNA molecules at a temperature between 65°C and 75°C (which encompasses the claimed range; page 32, lines 10-12).

With regard to claim 16, Richardson teaches each of these characteristics of the first heeled primer on page 39, lines 10-22:

In another aspect of the third embodiment of the present invention, the first heeled primer population consists of a population of nucleic acids comprising, from 5' end to 3' end:

- (i) a heel sequence of 15 to 22 nucleotides in length which is not complementary to the first strand cDNA nor the mRNA molecules initially present in the sample;
- (ii) An option but preferably present RNA polymerase promoter site;
- (iii) an oligo dT sequence of 15 to 35 nucleotides in length; and
- (iv) a variable sequence of 2-4 nucleotides in length that can hybridize to a mRNA molecule at the 5' end of the poly-A tail thereof, wherein substantially every possible variable sequence combination is found in said first heeled primer population.

With regard to claims 17 and 18, Richardson teaches detection of the sequence of interest according to the recited methods (page 5, lines 11-23):

Increase the number of nucleotide sequences corresponding to the mRNA species present in a sample is intended to designate an increase in nucleotide sequence to obtain a number of copies which is sufficient to allow at least one of the following methods:

- (i) detection of the sequence of interest with specific oligonucleotide probes;
- (ii) amplification of the sequence of interest with specific oligonucleotide primers;
- (iii) cloning of the DNA molecules obtained in a replication and/or expression vector, or
- (iv) In vitro RNA transcription, either for hybridization assays or for further reverse transcription optionally using unlabelled or labeled substrates followed by gene specific PCR or hybridization.

With regard to claim 19, Richardson teaches an example in which the first heeled primer comprises a T7 promoter (page 55, lines 26-33) and the second heeled primer comprises a T3 promoter (page 56, lines 25-29).

With regard to claim 20, Richardson teaches production of RNA "run-offs" using T7 and T3 RNA polymerases (see pages 53-54 and especially page 57, lines 17-32).

Richardson does not teach that the reverse transcribing with the first and second heeled primers to produce double-stranded cDNA was performed in a single step, as recited in amended claim 1. Rather, Richardson teaches adding the second primer after the first strand cDNA was synthesized (e.g. page 26, line 31).

Gu teaches synthesizing first and second strand cDNA in a single step (e.g. paragraph [0010]; paragraphs [0167]- [0168]) using thermostable DNA polymerases with reverse transcriptase activity.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to carry out the first and second strand cDNA synthesis using the "single-pot" method of Gu when practicing the method of Richardson, since this would have been more efficient. Also, Gu teaches this method can be carried out at elevated temperatures, thus overcoming the problem of RNA secondary structure during reverse transcription (see paragraph [0004]).

Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over Richardson et al (WO 01/06004 A2, cited on the IDS of 8/28/2006) in view of Gu et al (US 2003/0180737) as applied to claims 1-6 and 8-19 above and further in view of Fend et al (American Journal of Pathology, vol 154, no 1, pp 61-66, 1999, prior art of record).

The teachings of Richardson and Gu have been discussed. These references do not teach using a sample in his method that was obtained by laser capture microdissection.

Fend teaches a method for laser capture microdissection to obtain single cells for mRNA analysis (see entire article). Fend teaches that analysis of gene expression in normal or pathologically altered cells can "lead to the establishment of genetic fingerprints of neoplasms" (page 61, 1st paragraph following abstract). Fend also teaches that microdissected samples allows the isolation of morphologically identified cell populations down to the single-cell level, which overcomes the problem of tissue heterogeneity that can confound the attempt to differentiate gene expression patterns

between neoplastic and non-neoplastic tissue (page 61, 1st and 2nd paragraphs following abstract).

It would have been *prima facie* obvious to use Richardson's method (modified according to the teachings of Gu) to analyze samples obtained by laser capture microdissection, since Fend clearly teaches the value of laser capture microdissected samples and since Richardson was clearly interested in analyzing the RNA content of single cells (e.g. see page 3, lines 26-29, page 43, lines 32-35, page 44, lines 34-35, page 45, lines 1-3, page 58, lines 11-16). Thus it would have been obvious to one of skill in the art at the time the invention of the instant application that Richardson's method was ideally suited for the analysis of laser capture microdissection samples.

Response to Arguments

Applicant's arguments filed 07/07/2008 have been fully considered but they are not persuasive.

Applicant argues that "it is necessary for the Patent Office to show that the ordinarily skilled artisan would have had some suggestion or motivation to combine the cited references" (page 6, last paragraph of the response filed 07/07/2008). It is respectfully submitted that the rejection states:

"It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to carry out the first and second strand cDNA synthesis using the "single-pot" method of Gu when practicing the method of Richardson, since this would have been more efficient. Also, Gu teaches this method can be carried out at

elevated temperatures, thus overcoming the problem of RNA secondary structure during reverse transcription (see paragraph [0004])."

This provides sufficient motivation.

Applicant discusses at length the examiner's mention of the *KSR* decision (*KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385). This case was cited by the examiner in passing to rebut the presumption that an explicit teaching, suggestion, or motivation *per se* is required for a *prima facie* case of obviousness. Applicant argues that "[t]he *KSR* decision is limited to simple mechanical inventions wherein a combination of elements that were well known in the art could easily be envisioned working together in a simple device" (pages 6-7 of the response). Notwithstanding this argument, with which the examiner does not agree, the issue is moot since a motivation was set forth in the rejection.

As to Applicant's argument that "the ordinary skilled artisan would not have been able to predict the outcome of combining multiple chemical reaction steps into a single step", it bears repeating what the actual difference is between the prior art (Richardson) and the method as claimed. The method as claimed carries out the synthesis of the 1st strand of cDNA and the 2nd strand of cDNA in a single step (see claim 1). In other words, both the forward and reverse primers were added and the reaction was carried out in a single tube (e.g. Example 1, page 5 of specification). In contrast, Richardson's disclosure indicates that the 2nd primer was added after the reaction containing the first primer had been incubated for a period of time (e.g. Richardson, page 26, line 31). So the difference is that rather than adding the second primer to the mixture *after* the first

strand of cDNA as synthesized, the claimed invention adds both primers to the tube *and then* carries out consecutive synthesis of the first and second strands. An ordinarily skilled artisan *would* have been able to predict the outcome of this alteration, because Gu had already shown that synthesis of both 1st and 2nd strands of cDNA in a continuous reaction could be achieved (e.g. paragraph [0168]: "In further embodiments, reverse transcription of an RNA into a cDNA, second strand synthesis of a copy of the cDNA, and amplification of the cDNA are conducted in a continuous process in the same reaction mix."). Therefore, Gu provides not only a motivation but also predictability. Thus, Applicant's argument that such success could not have been predicted is not persuasive.

Lastly, Applicant argues that the claimed invention produces unexpected advantages and results. First, Applicant argues that fewer amplification cycles are required as compared to the prior art method (page 8 of response). This allegation is also found in the specification as filed (paragraph bridging pages 1-2). Keeping in mind that the only difference between the method of Richardson and the claimed method is the performance of both 1st and 2nd strand cDNA synthesis in the same reaction, the examiner finds no comparative data in the disclosure as filed to indicate that this difference translates into fewer required cycles of amplification (or that the method as claimed produces more amplification products than the method of Richardson). Allegations of unexpected results must be supported by evidence. See MPEP 716.02-716.02(b).

Furthermore, other advantages argued by Applicant on page 8 of the response (avoiding need for rare restriction sites, allows inclusion of specific sites for lambda cloning) appear to have nothing to do with whether or not reverse transcription and amplification steps are performed as discrete or combined steps. In addition, these elements are not elements recited in the claims, and so do not distinguish over Richardson.

As to Applicant's remarks on page 8:

Another advantage is that the production of complex products is minimized, due in part to the use of unique sequences in the heeled 5'-amplification and the heeled 3'-amplification primers which are absent from the genome being investigated. Moreover, while the procedure described in the Richardson *et al.* publication uses a single primer to amplify the products after reverse transcriptase and second strand synthesis, the present invention provides the significant advantage that two separate primers of unique sequence are used.

This argument is not persuasive because Richardson clearly teaches this concept in, for instance, Example V, pages 55-58, wherein the primer sequences were chosen based on their absence from the genome being investigated (see page 55, lines 29-30 and page 56, lines 25-26). In addition, while there are examples in Richardson where the first and second reverse transcription primers had similar 5' tags such that a single complementary amplification primer was used in the subsequent PCR stage (page 55, Example V; see especially page 57, lines 5-6), Richardson *also* teaches at pages 31-32 (bridging paragraph) that amplification of the 1st and 2nd cDNA strands can be performed "with primers selected from the group consisting of (1) a primer comprising a portion of the heel sequence of the first heeled primer...(2) a primer comprising a portion of the heel sequence of the second heeled primer...and (3) a

mixture of primers (1) and (2)...". This clearly implies the use of 1st and 2nd reverse transcription primers with differing heel sequences, and corresponding use of different amplification primers complementary to the different heel sequences. Thus, this element, which is also not claimed, is also not distinguishing over Richards.

Conclusion

All claims are drawn to the same invention claimed in the application prior to the entry of the submission under 37 CFR 1.114 and could have been finally rejected on the grounds and art of record in the next Office action if they had been entered in the application prior to entry under 37 CFR 1.114. Accordingly, **THIS ACTION IS MADE FINAL** even though it is a first action after the filing of a request for continued examination and the submission under 37 CFR 1.114. See MPEP § 706.07(b). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire **THREE MONTHS** from the mailing date of this action. In the event a first reply is filed within **TWO MONTHS** of the mailing date of this final action and the advisory action is not mailed until after the end of the **THREE-MONTH** shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than **SIX MONTHS** from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAMUEL WOOLWINE whose telephone number is (571)272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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